UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
TRABALHO DE CONCLUSÃO DE CURSO

ADAPTATION OF RAPID RESAPOLYMYXIN *ACINETOBACTER/ PSEUDOMONAS* NP AS A FEASIBLE AND RAPID TEST TO DETECT POLYMYXIN B RESISTANCE

JULIANA LUNELLI GALVAN

Porto Alegre

2022

Juliana Lunelli Galvan

ADAPTATION OF RAPID RESAPOLYMYXIN *ACINETOBACTER/ PSEUDOMONAS* NP AS A FEASIBLE AND RAPID TEST TO DETECT POLYMYXIN B RESISTANCE

Trabalho de Conclusão de Curso apresentado ao Curso de Farmácia da Universidade Federal do Rio grande do Sul como requisito à obtenção do título de grau de Farmacêutico.

Orientadora Profª. Dra. Juliana Caierão

Porto Alegre

2022

DEDICATÓRIA

Dedico este trabalho aos meus pais, Angela Maria Lunelli Galvan e Edgar Luiz Galvan, por me proporcionarem condições de seguir almejando e realizando meus sonhos.

AGRADECIMENTO

Agradeço, primeiramente, ao meus pais Angela e Edgar, que sempre dedicaram todo o tempo e atenção possíveis para a minha educação, e para os meus sonhos. Não tenho palavras para agradecer todo o esforço que fazem até hoje para me proporcionar sempre o melhor e por todo incentivo, bençãos e orações destinadas a mim todos os dias. Espero conseguir retribuir todo o amor e dedicação da melhor forma.

À minha orientadora Prof. Dra. Juliana Caierão, por me receber tão bem em seu laboratório, acreditando no meu potencial e confiando em mim para fazer parte deste projeto. Agradeço por todo conhecimento compartilhado e por ser uma das responsáveis por plantar a sementinha da pesquisa e da bacteriologia em meu coração.

Ao meu namorado, Matheus Barazetti, por ser um dos meus maiores incentivadores, sempre me apoiando, torcendo por mim e comemorando cada pequena conquista. Obrigada por ser meu melhor "ouvido amigo" quando a distância impedia o abraço e apertava o peito. Falando em distância, serei eternamente grata por toda compreensão, amor e paciência durante todos esses anos.

Aos meus amigos e familiares, que não irei citar nomes, mas que sabem o quanto foram essenciais em todos os momentos da minha graduação. Agradeço por cada mensagem de preocupação, palavra de incentivo, gesto de carinho e cuidado. Vocês me ajudaram e muito, para que eu pudesse chegar até aqui.

Por último, gostaria de dedicar um espaço especial à minha amiga e coorientadora extraoficial Gabriela da Silva Collar. Agradeço por você ter sido a melhor influência que eu tive na universidade, desde o primeiro semestre até nossos últimos dias no laboratório, por me mostrar o mundo lindo (e algumas vezes traumático) da pesquisa, por me encorajar e não me deixar duvidar um dia sequer do meu potencial. Obrigada por todas as caronas, conselhos, aulas de excel e momentos inesquecíveis.

Muito obrigada.

APRESENTAÇÃO

Este trabalho foi elaborado em formato de artigo científico, de acordo com as normas da revista *Journal of Microbiological Methods*. Tais normas foram anexadas ao final do documento, para uma melhor leitura e compreensão da banca avaliadora.

Addaptation of Rapid ResaPolymyxin Acinetobacter/Pseudomonas NP® as a feasible and rapid test to detect polymyxin B resistance

Juliana Lunelli Galvan[1], Gabriela da Silva Collar[2], Juliana Caierão[1,2].

[1]Department of Analysis, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

[2]Post-Graduation Program of Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

[*]**Corresponding author:** Dr. Juliana Caierão, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul. Avenida Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brazil.

Phone: +555133032139; **Mobile:** +5551999863645

e-mail: juliana.caierao@ufrgs.br

Keywords: Polymyxin B; Rapid test; Bacterial resistance; Resazurin; Susceptibility, *A. baumannii*; *P. aeruginosa*

Abstract

Considering the need of rapidly determine polymyxin B susceptibility in carbapenem-resistant isolates, Rapid ResaPolymyxin *Acinetobacter/Pseudonomas* NP test was developed. In this study, we adapted the methodology for a reproducible in-house version, which presented excellent results for *Acinetobacter baumannii*. However, more adaptations for *Pseudomonas aeruginosa* became clearly necessary.

1. Introduction

It is well recognized that bacterial resistance is a matter of urgency and worldwide concern. Multidrug-resistant (MDR) bacteria are hazardous and need careful attention as they pose a particular risk in hospitals, nursing homes, and among patients considered to be at high risk for acquiring infections by these bacteria. In 2017, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) published a list containing the most worrying bacteria to human health; among them, at the top of the critical priority list, are *Acinetobacter baumannii* and *Pseudomonas aeruginosa* resistant to carbapenems (Fournier et al., 2006; Willyard, 2017).

Infections caused by these pathogens increase patient morbidity and mortality, and prolong hospital stay, increasing costs. Indeed, mortality rates of bloodstream infections caused by *A. baumannii*, as an example, vary between 30 to 52% (Dijkshoorn et al., 2007) and pneumonia caused by this bacteria has been highly related to patient mortality (Abbo et al., 2007; Falagas and Rafailidis, 2007; Fournier et al., 2006).

For years, treatment of infections caused by MDR *A. baumannii* and *P. aeruginosa* focused on the use of carbapenems. However, the emergence and spread of carbapenem-resistant bacteria has limited the effectiveness of these therapeutic schemes (Ibrahim et al., 2021). In these cases, few antimicrobial options are available; among them, polymyxins-centered treatments are widely used, mainly in settings where the new combinations of beta-lactam/beta-lactamase inhibitors are not largely available, or where costs are an issue (Sheu et al., 2019).

The clinical use of polymyxins (colistin and polymyxin B) was abandoned in the 1970s due to their toxic potential, mainly neurotoxicity and nephrotoxicity. However, with the emergence of MDR *A. baumannii* and *P. aeruginosa*, and later *Enterobacterales* resistant to carbapenems, its use was reassessed, and it is currently in the therapeutic pipeline (Poirel et al., 2017; Tsuji et al., 2019).

Although rates of polymyxin B resistance is low in most countries, it appears to be increasing due to selective pressure. Indeed, in some regions, polymyxins resistance is worryingly high, such as in Singapore, where it is as high as 50% (Chen et al, 2020; Lim et al., 2011).

In this scenario, the rapid and efficient detection of polymyxins susceptibility is crucial for both epidemiological monitoring and therapeutic management (Satlin, 2019). For this purpose, the gold standard method suggested by the CLSI and EUCAST is the is the determination of minimum inhibitory concentration (MIC) through broth microdilution (BMD). However, it is time consuming and may present interpretation issues, especially for *A. baumannii*, for which skipped-wells caused by polymyxins heterorresistance are common. Other methodologies, such as disk diffusion and concentration gradient strips (Etest®, bioMérieux), are not indicated due to high rates of false susceptibility, i.e. up to 32% (Tan and Ng, 2007).

Therefore, alternative shorter-time demanding methodologies have been evaluated, once it may affect patient outcome. Indeed, each hour of incorrect use of antibiotic therapy decreases chances of survival by 7.6% (Kumar et al., 2009), in addition to raising costs of treatment and hospital stay. Therefore, rapid susceptibility detection methods are desirable in order to optimize antibiotic stewardship and patient outcome.

For *Enterobacterales*, the Rapid Polymyxin NP test was proposed and has been extensively evaluated with good results (Nordmann et al., 2016; Jayol et al., 2016; Dalmolin et al., 2019; Shoaib et al., 2020; Collar et al., 2021). However, as it is based on the use of glucose, it is not applicable to *A. baumannii* and *P. aeruginosa*.

Recently, Lescat and coworkers (2019) developed an assay based on the reduction of blue-colored resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide) to resofurin, a pink-stained product, indicating the presence of metabolically active bacterial cells in the medium (Lescat et al., 2019). A commercial version of this test is now available, the RapidResa Polymyxin

Acinetobacter NP® (2019) test (Liofilchem, Roseto degli Abruzzi, Italy), which is based on the same protocol as its in-house version and was evaluated by Bouvier and coworkers (2021). Considering costs as an issue, we aimed to evaluate the effectiveness and reproducibility of the in-house format of the technique among *A. baumannii* and *P. aeruginosa*.

2. Materials and methods

2.1 Bacterial strains and polymyxin B susceptibility

Ninety-four carbapenem-resistant isolates (56 *A. baumannii* and 38 *P. aeruginosa*), consecutively recovered from patients attended in a hospital of Porto Alegre, south Brazil, were included. The study was approved by the local Research Ethics Committee. *P. aeruginosa* ATCC 27853 (MIC 0.5-2 μg/mL) and an *A. baumannii* from our personal collection, previously characterized as susceptible to polymyxin B through BMD (NF 278, MIC 0.25μg/mL) was used as negative (susceptible) control. Moreover, another *A. baumannii* from our personal collection, previously characterized as resistant to polymyxin B through BMD (NF 281, MIC 8 μg/mL) was included as positive (resistant) control.

Polymyxin B BMD was performed for all isolates to determine MIC. Results were interpreted according to BrCAST and EUCAST guidelines (Brazilian Committee on Antimicrobial Susceptibility Testing/European Committee on Antimicrobial Susceptibility Testing, 2022).

2.2 Rapid ResaPolymyxin *Acinetobacter/Pseudomonas* NP test.

Rapid ResaPolymyxin *Acinetobacter*/*Pseudomonas* NP (RRP-NP) test was performed according to Lescat and coworkers (Lescat et al., 2019). Briefly, a bacterial inoculum was added to a 96-well plate containing culture medium with and without polymyxin B (3.75 μg/mL) and the resazurin is added in due course, after an incubation period (Fig. 1).

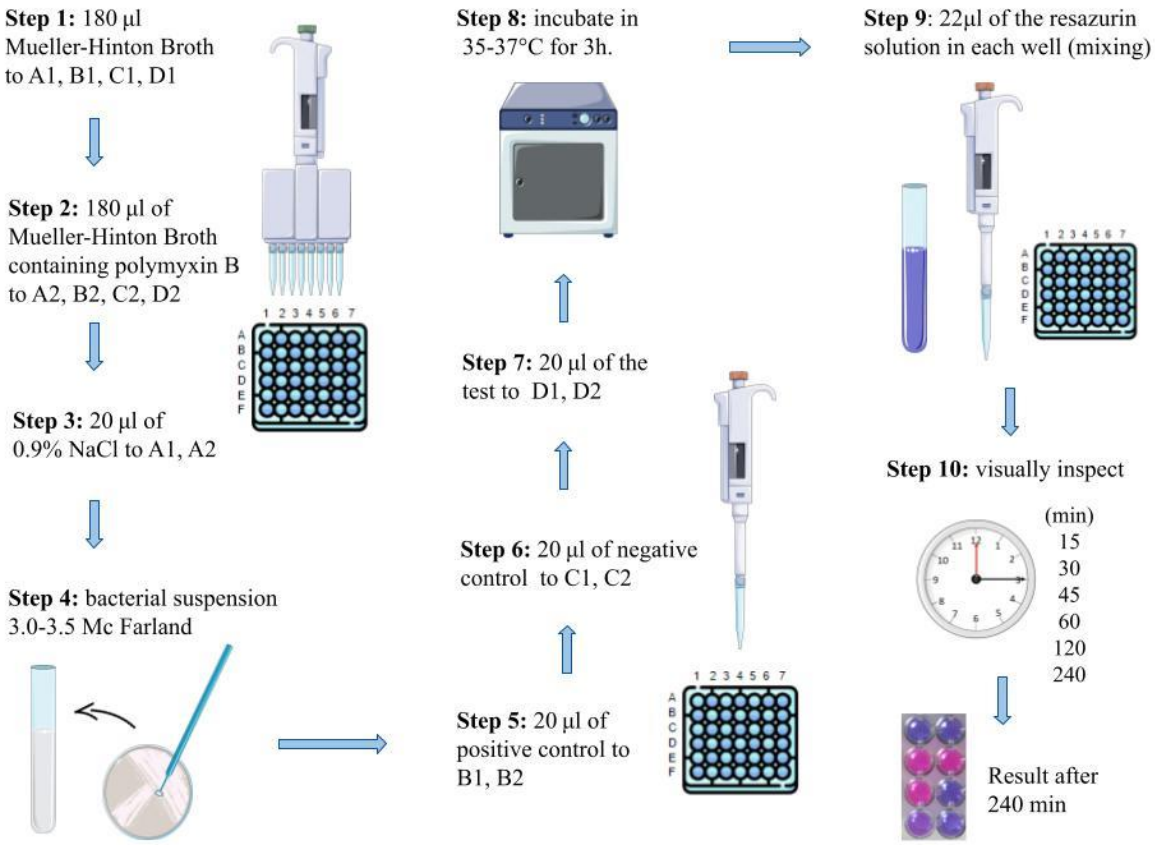

Figure 1: Methodology of the standardized version of the Rapid ResaPolymyxin *Acinetobacter/Pseudomonas* NP test.; The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

However, some important modifications were tested to adapt methodology to our laboratory and to make it reproducible. We tested three different media for culture bacterial: Mueller-Hinton agar (Sigma-Aldrich, USA), MacConkey agar (Kasvi, BR) and tryptic soy agar (TSA; Kasvi, BR), with the same incubation time and temperature, in order to understand if the pH of primary medium would interfere in the test.

Considering resazurin solution, we used pure resazurin rather than the commercial dye (Presto Blue™) as previously published, in order to reduce costs. Resazurin was solubilized in different solvents: sterile ammonium hydroxide and sterile distilled water. We evaluated concentrations of 0.2% and 0.02% to define the one that would be most suitable and allow the

shortest reading time. After preparation, resazurin solution was filtered through a 0.22 µm PES membrane filter and vial was conserved protected from light.

Bacterial suspensions (3.0-3.5 McFarland) were transferred to wells containing Mueller-Hinton broth (MHB, pH around 7; Sigma-Aldrich, USA), MHB pH 6.5 (adjusted with HCl), brain heart infusion Broth (BHI, Merck, GER), and a combined medium containing 50% BHI and 50% MH, in order to identify any possible interference in time needed for bacteria growth.

Finally, besides adding resazurin after 3h of incubation (as published elsewhere)(Lescat et al., 2019), we tried to decrease test turnaround time, adding the dye right after pipetting the inoculum, reducing 3h to reach results.

After the addition of resazurin, plate was visually observed every 15 min for 3h. Alteration of color from blue to purple/pink indicated positive result (resistant isolate) (Fig. 2). If color change was not obvious, result was considered inconclusive and repeated.

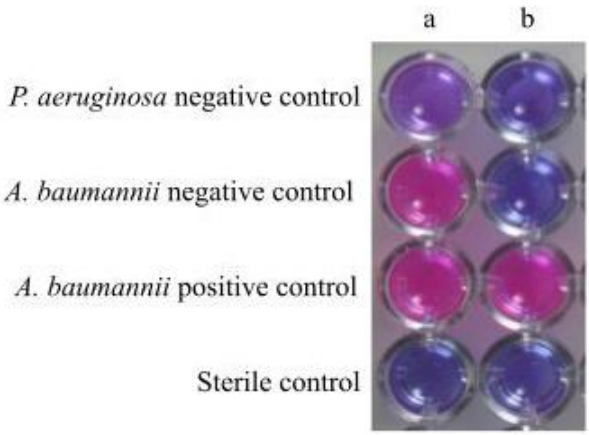

Figure 2: Results of the standardized version of Rapid ResaPolymyxin *Acinetobacter/Pseudomonas* NP test. Lines: *P. aeruginosa* ATCC 27853 *(*negative control); *A. baumannii* NF278 (negative control); *A. baumannii* NF281 (positive control); sterility control. Columns: (a) without polymyxin B; (b) with polymyxin B.

3. Results and discussion

After analyzing all variables, test was standardized as follows: Bacteria were cultured on TSA, as no difference was observed when changing the medium. Indeed, the use of BHI caused an unacceptable number of false positives (Fig.3.C). Resazurin was diluted on sterile distilled

water in a concentration of 0.02%. When diluting resazurin powder in ammonium hydroxide (Fig. 3.A), time needed for visualization of resazurin reduction was longer, being 2 hours after the addition of the resazurin. However, using sterile distilled water, 30 minutes after the addition of the dye color change was visible. Of note, Lescat and coworkers (2019) observed the beginning of color change after 15 minutes of incubation.

Acidification of the medium caused several false positive results. Indeed, resazurin is also used as a pH indicator (from orange [pH 3.5], to pink, to blue [pH>6.5]); then, when we modified pH of the medium, all the wells turned pink, including negative and sterility control (Fig. 3.B).

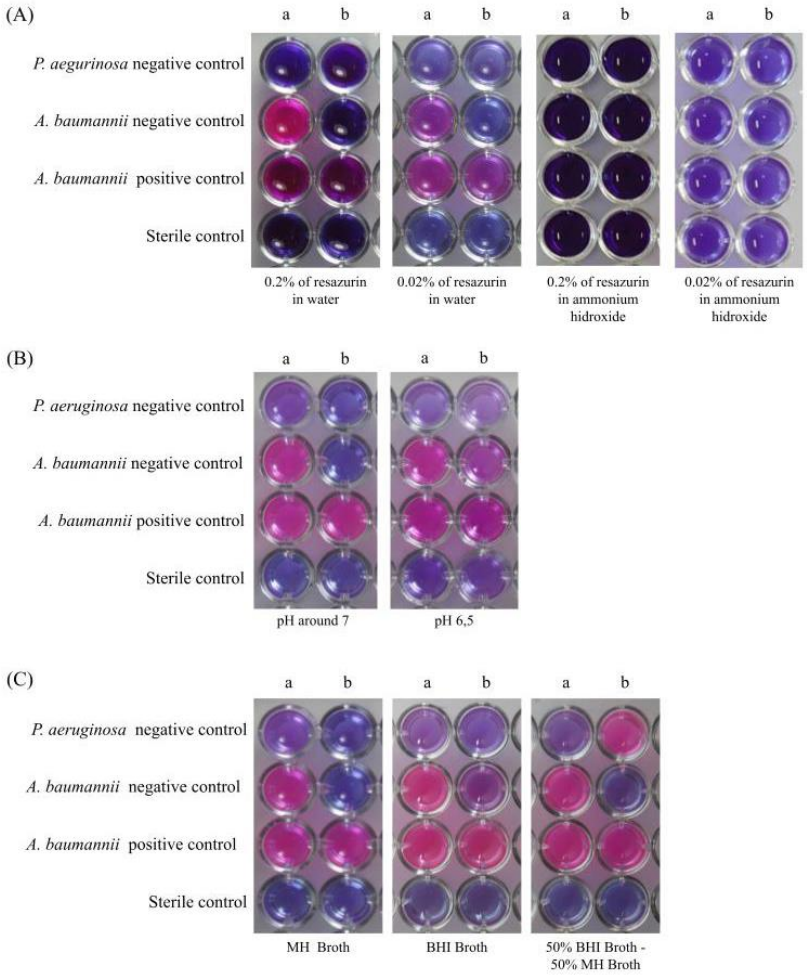

Figure 3: Results observed using different variables. Lines: *P. aeruginosa* ATCC 27853 *(*negative control); *A. baumannii* NF278 (negative control); *A. baumannii* NF281 (positive control); sterility control. Columns: (a) without polymyxin B; (b) with polymyxin B.

We tested polymyxin B rather than colisitin because it is widely used in Brazil. In addition, even though polymyxin B and colistin molecules differ by only one amino acid in the heptapeptide ring, they are different drugs in terms of pharmacokinetics/pharmacodynamics. Some data suggest that administration of colistin, in the form of the prodrug colistimethate, which might cause an increased risk of acute kidney injury when compared to polymyxin B (Ngamprasertchai et al., 2018). This may justify the choice of some institutions to preferentially use polymyxin B instead of colistin. Furthermore, several countries only have commercial access to polymyxin B (Humphries et al., 2019).

According to BMD, 14.9% (14/94) were resistant to polymyxin B, while 85.1% (80/94) were susceptible to this antimicrobial. Overall, MICs ranged from ≤ 0.125 μg/mL to >64 μg/mL, with 13.8% (13/94) of isolates presenting borderline MICs (2 or 4 μg/mL), as shown in table 1.

We observed 3.2% (3/94) of inconclusive results (i.e., no clear color change from blue to purplue/pink), all *P. aeruginosa* with MIC of 0.5 μg/mL). Of note, to evaluate performance of the test, these 3 results were disregarded. Overall, RRP-NP demonstrated a categorical agreement (CA) of 91.2% (83/91) among our bacterial population. When comparing with BMD, RRP-NP correctly identified 75 out of 77 isolates susceptible to polymyxin B, with a specificity of 97.4%, a positive predictive value (PPV) of 80% (8/10), and 2.2% (2/91) of major error (ME). The 2 false positive isolates were *P. aeruginosa* (MICs of 1 μg/mL) which turned positive (purple/pink) within 60 min of incubation. On the other hand, RRP-NP adequately characterized 8 out of the 14 isolates resistant to polymyxin B: 57.1% of sensitivity, negative predictive value (VPN) of 92.6% (75/81), and 6.6% (6/91) of very major error (VME). Six *A. baumannii* presented false negative results (MICs of 4 (n=2), 8 (n=1), 16 (n=1), 32 (n=2) μg/mL).

Lescat and coworkers evaluated 43 *A. baumannii* (30.2% (13/43) resistant to colistin) and 49 *P. aeruginosa* (20.4% (10/49) resistant to colistin). They observed sensitivity of 100% and specificity of 97% with three isolates incorrectly characterized as resistant by RRP-NP (MICs of

1 [n=1] and 0.25 µg/ml [n=2]). Our sensitivity was much lower than previously published. However, it must be highlighted that of the 6 false negative results, 2 presented borderline MIC (4 µg/mL).

Some important issue was observed among *P. aeruginosa*. In order to obtain a complete color change (pink), a very long incubation time was necessary (more than 6 hours). That was the reason why, for *P. aeruginosa*, we assumed as positive a clear color change from blue to purple, which was possible to observe after 4h of experiment (3h of incubation plus 1h after addition of resazurin).

Germ et al. (2019) and Jia et al. (2020) evaluated RRP-NP among *A. baumannii*. Jia et al. (2020) found sensitivity and specificity of 100 and 96%, respectively, which is in accordance of what was found by Germ and coworkers (2019): 93.3% of sensitivity and 93.3% of specificity, with 92.3% of PPV. We believe that the fact they evaluated exclusively *A. baumannii* justify better results of sensitivity and specificity compared to ours, as we observed more accurate results among this specie. Indeed, false positive results were observed among *P. aeruginosa*.

Bouvier et al. (2021), evaluating the commercial version of the test, observed 96% of sspecificity and 97% of sensitivity when analyzing 62 *A. baumannii* (36 susceptible to colistin [MICs from <0.125 to 1 mg/L] and 26 resistant [MICs from 4 to >128 mg/L]). They observed only one false positive result (MIC of 0.25 mg/L) and one false negative result (MIC of 16 mg/L).

Our values of specificity and, mostly, sensitivity were lower than previously reported. It is well recognized the influence of small sample size over these parameters. Indeed, this is the most notorious limitation of our study, as only 14 isolates resistant to polymyxin B could be analyzed. We strongly believe increasing sample size would improve sensitivity of the proposed methodology.

4. Conclusion

Despite the need to adjust many variables to obtain an in-house reproducible RRP-NP, the methodology seemed to be promising for qualitatively detect polymyxin resistance among *P. aeruginosa* and *A. baumannii*, being faster and less laborious than BMD. The small sample size of our study significantly compromised results, so we have a future perspective to increase the number of tested isolates, including more isolates resistant to polymyxins.

5. References

Abbo, A., Carmeli, Y., Navon-Venezia, S., Siegman-Igra, Y., Schwaber, M.J., 2007. Impact of multi-drug-resistant Acinetobacter baumannii on clinical outcomes. Eur. J. Clin. Microbiol. Infect. Dis. 26, 793–800. https://doi.org/10.1007/s10096-007-0371-8

Chen, X., Xu, J., Zhu, Q., Ren, Y., Zhao, L., 2020. Polymyxin B resistance rates in carbapenem-resistant Pseudomonas aeruginosa isolates and a comparison between Etest® and broth microdilution methods of antimicrobial susceptibility testing. Exp. Ther. Med. 20, 762–769. https://doi.org/10.3892/etm.2020.8777

Dijkshoorn, L., Nemec, A., Seifert, H., 2007. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat. Rev. Microbiol. 5, 939–951. https://doi.org/10.1038/nrmicro1789

Falagas, M.E., Rafailidis, P.I., 2007. Attributable mortality of Acinetobacter baumannii: no longer a controversial issue. Crit. Care 11, 134. https://doi.org/10.1186/cc5911

Fournier, P.E., Richet, H., Weinstein, R.A., 2006. The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities. Clin. Infect. Dis. 42, 692–699. https://doi.org/10.1086/500202

Ibrahim, S., Al-Saryi, N., Al-Kadmy, I.M.S., Aziz, S.N., 2021. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Mol. Biol. Rep. 48, 6987–6998. https://doi.org/10.1007/s11033-021-06690-6

Kumar, Anand, Ellis, P., Arabi, Y., Roberts, D., Light, B., Parrillo, J.E., Dodek, P., Wood, G., Kumar, Aseem, Simon, D., Peters, C., Ahsan, M., Chateau, D., 2009. Initiation of Inappropriate Antimicrobial Therapy Results in a Fivefold Reduction of Survival in Human Septic Shock. Chest 136, 1237–1248. https://doi.org/10.1378/chest.09-0087

Lescat, M., Poirel, L., Tinguely, C., Nordmann, P., 2019. A Resazurin Reduction-Based Assay for Rapid Detection of Polymyxin Resistance in Acinetobacter baumannii and Pseudomonas aeruginosa. J. Clin. Microbiol. 57, e01563-18. https://doi.org/10.1128/JCM.01563-18

Lim, T.-P., Lee, W., Tan, T.-Y., Sasikala, S., Teo, J., Hsu, L.-Y., Tan, T.-T., Syahidah, N., Kwa, A.L., 2011. Effective Antibiotics in Combination against Extreme Drug-Resistant Pseudomonas aeruginosa with Decreased Susceptibility to Polymyxin B. PLoS ONE 6, e28177. https://doi.org/10.1371/journal.pone.0028177

Moreira, N.K., Caierão, J., 2021. Ceftazidime-avibactam: are we safe from class A carbapenemase producers' infections? Folia Microbiol. (Praha) 66, 879–896. https://doi.org/10.1007/s12223-021-00918-5

Neto, J. de R.B.J., Reis, F.B., Desiderio, M. de M., Romero, M.G. de V., Aguiar, M.F. de C., Pimentel, I.D.S., Filho, D.F. de F., Cavalcante, A.C.O., Cavalcante, G.O., Santos, F., Barbosa, L.P., Mota, A.C.L., Coelho, R.V., Medeiros, M.S., 2022.

ENFRENTAMENTO DE SURTO POR SUPERBACTÉRIAS EM MEIO AO AUMENTO DA COVID-19 EM 2021. Braz. J. Infect. Dis. 26, 101944. https://doi.org/10.1016/j.bjid.2021.101944

Poirel, L., Jayol, A., Nordmann, P., 2017. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin. Microbiol. Rev. 30, 557–596. https://doi.org/10.1128/CMR.00064-16

Satlin, M.J., 2019. The Search for a Practical Method for Colistin Susceptibility Testing: Have We Found It by Going Back to the Future? J. Clin. Microbiol. 57, e01608-18. https://doi.org/10.1128/JCM.01608-18

Sheu, C.-C., Chang, Y.-T., Lin, S.-Y., Chen, Y.-H., Hsueh, P.-R., 2019. Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options. Front. Microbiol. 10, 80. https://doi.org/10.3389/fmicb.2019.00080

Tan, T.Y., Ng, S.Y., 2007. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. Clin. Microbiol. Infect. 13, 541–544. https://doi.org/10.1111/j.1469-0691.2007.01708.x

Tsuji, B.T., Pogue, J.M., Zavascki, A.P., Paul, M., Daikos, G.L., Forrest, A., Giacobbe, D.R., Viscoli, C., Giamarellou, H., Karaiskos, I., Kaye, D., Mouton, J.W., Tam, V.H., Thamlikitkul, V., Wunderink, R.G., Li, J., Nation, R.L., Kaye, K.S., 2019. International Consensus Guidelines for the Optimal Use of the Polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). Pharmacother. J. Hum. Pharmacol. Drug Ther. 39, 10–39. https://doi.org/10.1002/phar.2209

Collar, G. da S., Raro, O.H.F., da Silva, R.M.C., Vezzaro, P., Mott, M.P., Cunha, G.R. da, Riche, C.V.W., Dias, C., Caierão, J., 2021. Polymyxin NP tests (from colonies and directly from blood cultures): accurate and rapid methodologies to detect polymyxin B susceptibility among Enterobacterales. Diagn. Microbiol. Infect. Dis. 99, 115264. https://doi.org/10.1016/j.diagmicrobio.2020.115264

Dalmolin, T.V., Dias, G.Á., de Castro, L.P., Ávila, H., Magagnin, C.M., Zavascki, A.P., de Lima-Morales, D., Barth, A.L., 2019. Detection of Enterobacterales resistant to polymyxins using Rapid Polymyxins NP test. Braz. J. Microbiol. 50, 425–428. https://doi.org/10.1007/s42770-019-00053-x

European Committee on Antimicrobial Susceptibility Testing, n.d. Breakpoint tables for interpretation of MICs and zone diameters - Version 12.0. 2022.

Fournier, P.E., Richet, H., Weinstein, R.A., 2006. The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities. Clin. Infect. Dis. 42, 692–699. https://doi.org/10.1086/500202

Humphries, R.M., Green, D.A., Schuetz, A.N., Bergman, Y., Lewis, S., Yee, R., Stump, S., Lopez, M., Macesic, N., Uhlemann, A.-C., Kohner, P., Cole, N., Simner, P.J., 2019. Multicenter Evaluation of Colistin Broth Disk Elution and Colistin Agar Test: a Report from the Clinical and Laboratory Standards Institute. J. Clin. Microbiol. 57, e01269-19. https://doi.org/10.1128/JCM.01269-19

Jayol, A., Dubois, V., Poirel, L., Nordmann, P., 2016. Rapid Detection of Polymyxin-Resistant Enterobacteriaceae from Blood Cultures. J. Clin. Microbiol. 54, 2273–2277. https://doi.org/10.1128/JCM.00918-16

Ngamprasertchai, T., Boonyasiri, A., Charoenpong, L., Nimitvilai, S., Lorchirachoonkul, N., Wattanamongkonsil, L., Thamlikitkul, V., 2018. Effectiveness and safety of polymyxin B for the treatment of infections caused by extensively drug-resistant Gram-negative

bacteria in Thailand. Infect. Drug Resist. Volume 11, 1219–1224. https://doi.org/10.2147/IDR.S169939

Nordmann, P., Jayol, A., Poirel, L., 2016. Rapid Detection of Polymyxin Resistance in Enterobacteriaceae. Emerg. Infect. Dis. 22, 1038–1043. https://doi.org/10.3201/eid2206.151840

Shoaib, M., Hussain, A., Satti, L., Hussain, W., Zaman, G., Hanif, F., 2020. Evaluation of rapid polymyxin Nordmann Poirel test for detection of polymyxin resistance in clinical isolates of Enterobacteriaceae. Eur. J. Clin. Microbiol. Infect. Dis. 39, 2195–2198. https://doi.org/10.1007/s10096-020-03942-4

Willyard, C., 2017. The drug-resistant bacteria that pose the greatest health threats. Nature 543, 15–15. https://doi.org/10.1038/nature.2017.21550

Tables

Table 1: Results of RRP-NP for the detection of susceptibility to polymyxin B among *A. baumannii* and *P. aeruginosa.*

Isolate	Specie	MIC[a] polymyxin B (µg/mL)	RRP-NP	
			Result	Positivity Time (min)
NF345	*A. baumannii*	>64	P	30
NF337	*A. baumannii*	64	P	30
NF331	*A. baumannii*	32	N	-
NF384	*A. baumannii*	32	N	-
NF316	*A. baumannii*	16	P	30
NF398	*A. baumannii*	16	N	-
NF281	*A. baumannii*	8	P	30
NF286	*A. baumannii*	8	P	30
NF310	*A. baumannii*	8	N	-
NF284	*A. baumannii*	4[b]	N	-
NF296	*A. baumannii*	4[b]	P	30
NF326	*A. baumannii*	4[b]	N	-
NF341	*P. aeruginosa*	4[b]	P	75
NF388	*P. aeruginosa*	4[b]	P	90
NF285	*A. baumannii*	2[b]	N	-
NF289	*A. baumannii*	2[b]	N	-

NF308	*A. baumannii*	2[b]	N	-
NF318	*A. baumannii*	2[b]	N	-
NF330	*A. baumannii*	2[b]	N	-
NF335	*P. aeruginosa*	2[b]	N	-
NF397	*A. baumannii*	2[b]	N	-
NF402	*P. aeruginosa*	2[b]	N	-
NF279	*A. baumannii*	1	N	-
NF283	*A. baumannii*	1	N	-
NF297	*P. aeruginosa*	1	N	-
NF300	*A. baumannii*	1	N	-
NF301	*A. baumannii*	1	N	-
NF303	*P. aeruginosa*	1	N	-
NF304	*A. baumannii*	1	N	-
NF307	*P. aeruginosa*	1	N	-
NF311	*P. aeruginosa*	1	N	-
NF314	*A. baumannii*	1	N	-
NF319	*A. baumannii*	1	N	-
NF329	*P. aeruginosa*	1	N	-
NF332	*A. baumannii*	1	N	-
NF334	*P. aeruginosa*	1	N	-
NF339	*P. aeruginosa*	1	N	-
NF342	*A. baumannii*	1	N	-
NF344	*A. baumannii*	1	N	-
NF383	*A. baumannii*	1	N	-
NF386	*A. baumannii*	1	N	-
NF387	*A. baumannii*	1	N	-
NF389	*P. aeruginosa*	1	P	60
NF390	*P. aeruginosa*	1	N	-
NF391	*P. aeruginosa*	1	N	-

NF393	*P. aeruginosa*	1	N	-
NF400	*P. aeruginosa*	1	P	60
NF401	*P. aeruginosa*	1	N	-
NF403	*P. aeruginosa*	1	N	-
NF405	*A. baumannii*	1	N	-
NF280	*P. aeruginosa*	0.5	N	-
NF282	*A. baumannii*	0.5	N	-
NF287	*P. aeruginosa*	0.5	I	-
NF288	*P. aeruginosa*	0.5	N	-
NF290	*A. baumannii*	0.5	N	-
NF295	*P. aeruginosa*	0.5	N	-
NF298	*A. baumannii*	0.5	N	-
NF299	*P. aeruginosa*	0.5	N	-
NF302	*P. aeruginosa*	0.5	N	-
NF305	*P. aeruginosa*	0.5	N	-
NF309	*A. baumannii*	0.5	N	-
NF312	*P. aeruginosa*	0.5	I	-
NF313	*P. aeruginosa*	0.5	N	-
NF323	*P. aeruginosa*	0.5	N	-
NF325	*A. baumannii*	0.5	N	-
NF328	*P. aeruginosa*	0.5	I	-
NF333	*P. aeruginosa*	0.5	N	-
NF336	*P. aeruginosa*	0.5	N	-
NF338	*P. aeruginosa*	0.5	N	-
NF340	*P. aeruginosa*	0.5	N	-
NF385	*A. baumannii*	0.5	N	-
NF392	*P. aeruginosa*	0.5	N	-
NF394	*A. baumannii*	0.5	N	-
NF395	*A. baumannii*	0.5	N	-

NF406	*A. baumannii*	0.5	N	-
NF407	*A. baumannii*	0.5	N	-
NF408	*A. baumannii*	0.5	N	-
NF409	*P. aeruginosa*	0.5	N	-
NF278	*A. baumannii*	0.25	N	-
NF291	*A. baumannii*	0.25	N	-
NF292	*P. aeruginosa*	0.25	N	-
NF293	*A. baumannii*	0.25	N	-
NF294	*A. baumannii*	0.25	N	-
NF315	*A. baumannii*	0.25	N	-
NF317	*A. baumannii*	0.25	N	-
NF320	*A. baumannii*	0.25	N	-
NF321	*A. baumannii*	0.25	N	-
NF322	*A. baumannii*	0.25	N	-
NF327	*P. aeruginosa*	0.25	N	-
NF343	*A. baumannii*	0.25	N	-
NF399	*A. baumannii*	0.25	N	-
NF404	*A. baumannii*	0.25	N	-
NF306	*A. baumannii*	≤0.125	N	-
NF324	*P. aeruginosa*	≤0.125	N	-

MIC, minimum inhibitory concentration; RRP-NP, Rapid ResaPolymyxin NP test; P, positive (resistant); N, negative (susceptible); I, inconclusive.

[a]MIC of polymyxin B determined by BMD

[b]MICs borderline

Anexo 1

JOURNAL OF MICROBIOLOGICAL METHODS

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

- **Description** **p.1**
- **Audience** **p.2**
- **Impact Factor** **p.2**
- **Abstracting and Indexing** **p.2**
- **Editorial Board** **p.2**
- **Guide for Authors** **p.4**

ISSN: 0167-7012

DESCRIPTION

The Journal of Microbiological Methods publishes scholarly and original articles, notes and review articles. These articles must include novel and/or state-of-the-art **methods**, or significant improvements to existing methods. Novel and innovative applications of current methods that are validated and useful will also be published. JMM strives for scholarship, innovation and excellence. This demands scientific rigour, the best available methods and technologies, correctly replicated experiments/tests, the inclusion of proper controls, calibrations, and the correct statistical analysis. The presentation of the data must support the interpretation of the method/approach.

All aspects of **microbiology** are covered, except virology. These include agricultural microbiology, applied and environmental microbiology, bioassays, bioinformatics, biotechnology, biochemical microbiology, clinical microbiology, diagnostics, food monitoring and quality control microbiology, microbial genetics and genomics, geomicrobiology, microbiome methods regardless of habitat, high through-put sequencing methods and analysis, microbial pathogenesis and host responses, metabolomics, metagenomics, metaproteomics, microbial ecology and diversity, microbial physiology, microbial ultra-structure, microscopic and imaging methods, molecular microbiology, mycology, novel mathematical microbiology and modelling, parasitology, plant-microbe interactions, protein markers/profiles, proteomics, pyrosequencing, public health microbiology, radioisotopes applied to microbiology, robotics applied to microbiological methods,rumen microbiology, microbiological methods for space missions and extreme environments, sampling methods and samplers, soil and sediment microbiology, transcriptomics, veterinary microbiology, sero-diagnostics and typing/ identification.

The editors, editorial board members and scholarly reviewers are active scientists with an immense amount of collective experience that is used during the review and revision stages of articles. We encourage the submission of proposals for scholarly reviews and specials issues on emerging microbiological methods that are central to advancing microbiological knowledge. We look forward to receiving your proposals and articles.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services .

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

AUDIENCE

All microbiologists, biotechnologists, and geneticists

IMPACT FACTOR

2021: 2.622 © Clarivate Analytics Journal Citation Reports 2022

ABSTRACTING AND INDEXING

BIOSIS Previews
Embase
Pascal Francis
Current Contents
Web of Science
Elsevier BIOBASE
PubMed/Medline
UnCover
CAB International
Global Health
FSTA (Food Science and Technology Abstracts)
Current Biotechnology Abstracts
TOXFILE
Science Citation Index Expanded
Scopus

EDITORIAL BOARD

R. A. Guy, Guelph, Ontario, Canada
M.E. Hart, Jefferson, Arkansas, United States of America
H. Hendrickson, Palmerston North, New Zealand
J. Higgins, Beltsville, Maryland, United States of America
T. Hsiang, Guelph, Ontario, Canada
E.S. Kaneshiro, Cincinnati, Ohio, United States of America
M. Karrasch, Jena, Germany
G.A. Köhler, Tulsa, Oklahoma, United States of America
D.R. Korber, Saskatoon, Saskatchewan, Canada
M.P. Kozar, Silver Spring, Maryland, United States of America
H.C. Lai, Taoyuan, Taiwan
P. Lau, Montreal, Quebec, Canada
J.R. Lawrence, Saskatoon, Saskatchewan, Canada
J.O. Lay, Fayetteville, Arkansas, United States of America
K.P. Leung, Great Lakes, Illinois, United States of America
L. Lindler, Bethesda, Maryland, United States of America
E. Lutter, Stillwater, Oklahoma, United States of America
T McAllister, Ottawa, Ontario, Canada
M. Minnick, Missoula, Montana, United States of America
S. Mitarai, Tokyo, Japan
J. Mosher, Huntington, West Virginia, United States of America
N. Mykytczuk, Sudbury, Ontario, Canada
H. Nakatsu, West Lafayette, Indiana, United States of America
D. Naumann, Berlin, Germany
P.A. Noble, Seattle, Washington, United States of America
C.R. Osorio, Santiago de Compostela, Spain
van Overbeek, Wageningen, Netherlands
O.A. Oyarzabal, South Burlington, Vermont, United States of America
R.J. Palmer, Jr., Bethesda, Maryland, United States of America
M.W.J. van Passel, Wageningen, Netherlands
M. A. Patrauchan, Stillwater, Oklahoma, United States of America
C. Pavia, Old Westbury, New York, United States of America
M. Prieto, Leon, Spain
M. Rautenbach, Stellenbosch, South Africa
D. Ren, Rochester, New York, United States of America
M. Ridell, Goteborg, Sweden
J.R. Rose, Columbia, South Carolina, United States of America
R.F. Schell, Madison, Wisconsin, United States of America
L. Schena, Reggio, Italy
D. Schofield, Charleston, South Carolina, United States of America
R. Seviour, Bundoora, Victoria, Australia
H.M. Shapiro
A. Soares Rosado, Rio de Janeiro, Brazil
G.C. Stewart, Columbia, Missouri, United States of America
L. Suh, Auburn, Alabama, United States of America
R. Tenreiro, Lisbon, Portugal
E. Trees, Atlanta, Georgia, United States of America
O. Tresse, Nantes, France
G. Ulet, Southport, Queensland, Australia
J.D. Van Elsas, Haren, Netherlands
K. Waites, Birmingham, Alabama, United States of America
K. Warriner, Guelph, Ontario, Canada
A. Whiteley, Wallingford, United Kingdom
J. Withey, Detroit, Michigan, United States of America
D. Wunschel, Richland, Washington, United States of America
Y. Yang, Beltsville, Maryland, United States of America

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

The journal publishes full-length articles describing original work, notes (short concise communications), and review articles on methods, including their development, applicability, and present status. Microbiologists are encouraged to submit to the editors product information and a summary of articles published elsewhere with relevance to the readers of the journal. This information is subject to editorial approval. Articles describing the use of computers with software description are welcome. Submissions of a paper to this journal is understood to imply that it has not previously been published and that it is not being considered for publication elsewhere.

Types of paper
Full length Original Research Articles: Describing original work.

Review Articles: Reviews that do not contain experimental (hands-on) establishment of a method, but instead concern a summary or an evaluation of previously described methods, are welcome. Generally, the author should contact an editorial board member or editor familiar with the area who would then act as a sponsor. The name and address of the sponsor should be listed on the front page of the manuscript and will appear in the front page of the published article. Unsolicited articles may be sent to an Editor or Editor-in-Chief but it is preferred that approval of the topic area is obtained before the review is written.

Short Communications: A " Short Communication " is intended for the presentation of brief observations that do not warrant full-length papers. A Short Communication should be brief descriptions of novel apparatus or techniques, requiring real ingenuity on the author's part, that offer definite advantages over those already available. The abstract should be no more than 50 words, have no section headings in the body and report methods, results, and discussion in a single section. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum (2-3 maximum). Materials and methods should be described in the text, not in figure legends or table footnotes. Acknowledgments should be presented as in full-length papers, but no separate heading is used. The References section is identical to the **full-length papers**.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations

• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Language and language services
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors whose primary language is not English, are strongly encouraged to submit manuscripts after their being edited for correct English. This will speed up and increase the likelihood of acceptance of an article during the reviewing process. Authors who require information about language editing and copyediting services pre- and post-submission please visit https://webshop.elsevier.com/language-editing-services/language-editing/ or our customer support site at https://service.elsevier.com for more information.

Ethics in publishing
Please see our information on Ethics in publishing.

Declaration of competing interest
All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors should complete the declaration of competing interest statement using this template and upload to the submission system at the Attach/Upload Files step. **Note: Please do not convert the .docx template to another file type. Author signatures are not required.** If there are no interests to declare, please choose the first option in the template. More information.

Submission declaration and verification
Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language
Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We

suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

PREPARATION

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

•Please ensure your paper has consecutive line numbering - this is an essential peer review requirement.

•Also the manuscript should be double line spaced

Figures and tables embedded in text
Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary
Please supply, as a separate list, the definitions of field-specific terms used in your article.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

For 'Notes' Abstract should be no longer than 50 words

Graphical abstract
Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

US or GB spelling 3-6 (Alphabetized)

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Image manipulation
Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.

• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Text graphics
Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX and have such features embedded in the text, these can be left. See further under Electronic artwork.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

Tables should be compiled on separate pages with a short descriptive title, and legend if required, and numbered consecutively.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references
Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

References should be listed by author name and year of publication in parentheses in the body of the text [i.e.(Crissman and Hadley, 1995) two authors; (Crissman et al., 1998) more than two authors} and listed alphabetically in the reference section. References to journals should contain the names and initials of all authors, the year of publication, the title of the paper, the title of the periodical abbreviated according to List of Serial Title Word Abbreviations (available from the International Serials Data System, 20 rue Bachaumont, 75002 Paris, France. ISBN 2-904938-02-8), the volume number, and page number. References to books should also include the title of the book, the editors, and publishers. See examples below.

Schüler, D., Uhl, R., Bäuerlein, E., 1995. A simple light scattering method to assay magnetism in *Magnetospirillum gryphiswaldense*. FEMS Microbiol. Lett. 132, 139--145.

Tyssen, P., 1993. Hybridization with nucleic acid probes. In: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24, Elsevier, Amsterdam, pp. 375-436.

Shockman, G.D., Höltje, J.-V., 1994. Microbial peptidoglycan (murein) hydrolase. In: Ghuysen, J.-M.,Hakenbeck, R.(Eds.), Bacterial Cell Wall. Elsevier, Amsterdam, pp. 131- 166.

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.
Reference to software:
Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into a data article published in *Data in Brief*. A data article is a new kind of article that ensures that your data are actively reviewed, curated, formatted, indexed, given a DOI and made publicly available to all upon publication (watch this video describing the benefits of publishing your data in *Data in Brief*). You are encouraged to submit your data article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed, published open access and linked to your research article on ScienceDirect. Please note an open access fee is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your *Data in Brief* data article.

Data statement
To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

AFTER ACCEPTANCE

Online proof correction

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published.